TRANSLATION NO. 253

DATE: July 1968

DDC AVAILABILITY NOTICE

[redacted]

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

#252

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Detection of Listerella Infection in Wild Rodents, Insectivora and Ixodes Ticks.

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Zhurnal Mikrobiologii. 22 (6): 67-71: 1951.

Listerellosis is a disease to which man and various domestic and wild animals are subject.

During a massive bacteriological investigation of wild animals and ticks for the purpose of detecting tularemia infection, one of the author's (Olsuf'ev's) attention was diverted to a different pathogenic causative agent, a gram-positive bacillus which now and then was isolated from dead or live rodents and insectivora, and in one instance was detected in Ixodes ticks. During the period from Nov 1946 through Sept 1948, six strains were isolated from the wild animals. For this, 21,243 wild animals were tested (11,256 of these were field mice). In 1958 one strain was isolated from ticks. In addition 5 more strains were isolated by a co-worker at the tularemia institute, P. N. Olagolevaia, who worked under our supervision; in this, 5,879 wild animals were tested (of these 4,721 were field mice). These strains were transferred to us and consequently we were able to investigate 12 strains in all. 10 of these strains were tested somewhat in detail, and in 2 strains the antigenic properties were chiefly investigated. All the strains proved to be completely identical to the causative agent of listerellosis - Listeria monocytogenes. For comparison we had two standard strains, Nos. 139 (rabbit) and 249 (mouse), which we received from Prof. P. P. Sakharov.

Of the 12 strains that were investigated 9 were isolated from rodents, of which 8 were field mice (Microtus arvalis) and one a water rat (Arvicola terrestris), and 2 from insectivora, of which one was a water shrew (Neomys fodiens) and one a common shrew (Sorrex araneus); one strain was isolated from pubescent ticks, Dermacentor pictus; 5 strains were isolated from dead animals and 6 from animals that were captured alive. The sick and dead animals were discovered both in winter and summer. These were more frequently sporadic cases, but at times group murrains were noted. In winter the animals were chiefly taken from straw stacks and in the summer from open stations. The greater number of finds of L monocytogenes (10 of 12) occurred in 1948 and the winter of 1948/49. Evidently this is explained by the fact that during this period of the investigations they began to pay more attention to the listerella infection in the laboratory. Instead of a direct bacteriological investigation of the organs of the procured animals, almost all the strains were received through biological means by the utilization of a subinoculation of the organs (spleens, lymphatic glands, bone marrow in special cases) into a sensitive animal, white mouse or field mouse, with a subsequent bacteriological investigation of the organs of the animals that died (the method used for mass analyses for tularemia).

Field mice (M. arvalis) were used in the subinoculation more frequently in those cases where the organs of wild animals that had died were being

investigated. First of all, the field mice were held in the laboratory for at least 2 weeks. This procedure, i.e. the subinoculation of organs from the dead field mice into fresh animals, permitted avoiding mistakes which may develop when working with white mice that have their own infections. Strains No. 1321, 243, 81 and 394 were isolated via the field mice.

The field mice died in time periods ranging from 72 to 144 hours with the following pathologic-anatomy changes: a dense infiltration with a necrotic or suppurative focus in the infected area; hyperemia of the vessels in the subcutaneous cellular tissue; enlargement of the liver with plural minute necrotic nodules; enlargement of the suprarenal glands and spleen, but without nodules; distension of the small intestine in the beginning portions (with a yellowish content). The white mice died within 120-240 hours and upon their autopsy, practically the same changes were detected as in the field mice, although there were not always nodules in the liver; in two mice, which had died after 192 and 240 hours, the spleen was sharply enlarged and covered with numerous necrotic nodules that had never been noted in the field mice. Svintsov (1948) notes that in his tests, necrotic nodules of the liver and spleen in white mice were noted in 50-60 % of the cases. A culture from the organs of the field mice and white mice that had died was easily obtained on a weakly alkaline meat-peptone agar. In smear impressions from the organs L. monocytogenes was detected in sufficiently large quantity; the bacilli were at times situated in the protoplasm of leukocytes. In 3 analyses (Nos. 153, 2026 and 3503) the infection only developed in a second subinoculation, while the animals (white mice) of the first subinoculation were sacrificed on the 10th day; in their organs were detected small changes which testified as to the presence of the infection: hyperemia of the vessels in the subcutaneous cellular tissue, and singular necrotic nodules in the liver and spleen etc. In these analyses 2 white mice and 1 field mouse of the second subinoculation died after 96-192 hours, with changes as described above, and from their organs were taken L. monocytogenes cultures.

Strain No. 2753 was taken directly from a dead water rat by means of a sowing from the liver onto a meat-peptone agar. Within 24 hours a growth of two causative agents was discovered, Listeria monocytogenes and Erysipelotarix rhusiopathiae, with a predominance of the former's colonies. The E. rhusiopathiae colonies were clearly distinguished by their smaller dimensions. Therefore, the water rat died from a combination of listerellosis and an erysipelatous infection. The rat's liver was sharply enlarged and threaded with numerous small necrotic nodules. The erisipelatous infection was encountered rather frequently among the rodents in the locale being investigated; up to 30 % of the water rats were affected by it (Olsuf'ev and Dunaeva).

It is of particular importance to dwell on the conditions of isolating strain No. 449 from the ticks. 1,000 pubescent ticks of various degrees of blood satiation were collected from cows that seemed to normal by exterior appearance. The ticks were divided into 10 groups, 100 to a group, and after grinding with a physiological solution in a mortar they were subcutaneously injected, each group separately, into 10 guinea pigs. One guinea pig died on the 9th sutki (24-hour period- Tr note); upon its autopsy the following was discovered: at the place of infection in the inguinal region, an encysted cavity containing a turbid-gray viscous substance; the inguinal glands were

enlarged to the size of peas, sharply hyperemic; the vessels of the subcutaneous cellular tissue are sharply hyperemic; as to the abdomen, beneath the skin is a small purulent-caseous area which is connected to the focus of inflamation in the inguinal region; the spleen is enlarged to twice its normal size, cherry colored, thickened, blood-filled, without nodules, very strongly enlarged, of a light-yellow-clay color, compact, rather dry in the cross section of the incision; lungs hyperemic; kidneys enlarged; suprarenal glands x sharply enlarged and hyperemic. During the microscopy of the glands, liver and spleen, a small number of small, narrow bacilli was detected in every field of vision. Within 24 hours after a sowing from the spleen and liver onto a meat-peptone agar, a profuse growth of a pure culture of L. monocytogenes was received.

Because guinea pigs have comparatively little sensitivity to listerellosis, one must assume that the ticks contained a large quantity of the pathogen.

The characteristics of the strains that were isolated proved to be as follows. The pathogen appears as a small rod, somewhat narrower and shorter than the bacilli of the intestinal-typhous group, but visibly larger than the pathogen of the erysipelatous infection. The bacillus is weakly motile, does not decolorize by Gram's method, stains well with aniline dyes. In agar cultures it grows as straight, short ovoid rods, at times nearly cocci, which on a smear form separately or in small heaps (in palisades). In a close grained egg-yolk medium the bacilli grow to the same dimensions as in agar. In that the listeria differs from erysipelothrix, which in an egg-yolk medium will grow in a form of longer rods and threads than in agar. In broth it will grow in form of straight, short rods or coccobacteria, which in a smear culture are formed separately, by pairs, or in small groups, and less frequently in short chains. It is easily grown in common weakly alkaline nutritional mediums, but the growth received is not profuse. In a meat-peptone broth, after a 24 hour growth at 37°, a weak uniform opacification is noticed, which correlates approximately to 400-500 million microbic cells per cm^3 (by the TsGNKI bacterial standard); a small sedimentation is formed on the bottom, which upon shaking rises from the bottom in a small queue; scum does not form and there is no ringing of the container's sides. On an agar slant, with a profuse inoculation, the growth after 24 hours is in the form of a semi-transparent, grayish colored application of numerous small colonies fused together and partially isolated only along the edges of the streak; the growth is seen well by the naked eye. The 24 hour old colonies in the dishes are small, circular, flt, with even edges, semi-transparent, weakly chromogenic in a passing light. In gelatin after several sutki (24-hour periods - Tr note) there was a growth along the puncture in the form of small granules; later, by approximately the 20th day, in some cases in the upper portion of the puncture may appear small clusters of minute, tomentose threads going off perpendicularly from the puncture, but they never attain the sumptuosity that is characteristic for gelatin cultures. The gelatin does not liquefy. In a Clark culture medium, after a 96 hour growth at 37°, the reaction with methyl red is positive (reddening of the medium). Biochemical activity was carried out in 10 of our strains and in the 2 standard strains. All strains after a 24-hour growth at 37° decomposed glucose and maltose to an acid, after 3-5 sutki (24-hour periods - Tr note) - lactose, and after 10-27 sutki - sucrose and glycerol; mannitol and dulcitol (?) were not fermented.

By morphological, tinctorial, cultural and biochemical properties all our strains proved to be completely identical to each other and corresponded in full to standard strains Nos. 139 and 249.

For the investigation of the strains' antigenic properties we resorted to the agglutination reaction. By means of a five-fold immunization of rabbits with heat-killed cultures, antiserums were received to 7 of our strains and to standard strain No. 139. The titers of the serums amounted to 1:1,600--1:3,200. The results of the cross agglutination are presented in table 1.

All investigated strains proved to be serologically identical to each other. The agglutination was completely clear - there was a complete clarification of the liquid and a precipitation of the clump to the bottom of the test tube in the form of a clearly outlined umbrella; when shaken, the clump rose from the bottom of the test tube in the form of minute strands of threads; with a protracted shaking the threads broke up into a homogenous cloud. In the serum's lower dilutions (1:50-1:100) there was a sedimentation of one degree or another and at times it was fully dissolved. Apparently the antiserums, together with the agglutinins, contained rather powerful bacteriolysins. Particularly the lysis was frequently noted in the tests of strain No. 1321 and its serum; this made it difficult to properly determine the terminal titers of the agglutination. This strain was partially dissolved even in the control. Possibly this was stipulated by aging of the strain, or by the presence of a bacteriophage. The strain was investigated 2½ years after the isolation. Strains No. 286 and 394 were agglutinated with antiserum No. 81 in a dilution of 1:1,600. Due to the fact that strain No 2753 was isolated from a water rat simultaneously with the strain E. rhusiopathiae, there was prepared for the latter a rabbit antiserum with a titer of 1:1600. A cross-agglutination test showed that even in a minimum dilution (1:50) the serums did not react with the heterologous strains.

All of the isolated strains proved to be pathogenic for field mice. With a subcutaneous injection of 0.1-0.2 cm^3 of a 24-hour broth culture the mice died after 48-96 hours with changes typical for listerellosis. Of particular interest are: the enlarged liver, without exception threzded with grayish-yellowish necrotic nodules, and enlarged suprarenal glands, which at times were one-third the length of the kidney. The changes were so characteristic that the infection of the mice began to be used by us in practice for diagnostic purposes in the preliminary investigation of the cultures being isolated.

(Table 1)

The degree of sensitivity of the field mice and white mice to listerellosis was more accurately determined in special tests. The infection with the three strains was carried out subcutaneously shortly after their isolation. The field mice infected were full grown, weighing 20-25 g.; the white mice weighed 12-16 g. In all there were 29 field mice and 20 white mice in the tests (table 2). The absolute lethal dose for the field mice and white mice equalled 100 million microbic cells. The greater portion of the animals died from 10 and 1 million cells, whereas many animals survived from 100 to 10 thousand. In those animals which died from listerellosis the pathological-anatomy changes were completely tupical for acute listerellosis, and the diagnosis in all cases was substantiated by a positive bacterial microscopy and

an isolation of a primary culture. The disease in the field mice and white mice took a course in the form of septicemia. In those white mice and field mice which survived, there was discovered a well expressed immunuty, after two months, to a control subcutaneous infection with a virulent strain in a dose known to be lethal. The strains proved bo be pathogenic for fabbits. After an intravenous injection of 0.5 cm^3 of a 24-hour broth culture, the rabbits died after 72 hours from acute listerellosis; in the liver was detected a great quantity of yellowish necrotic foci.

(Table 2)

In the process of isolation and investigation of the listeria strains we encountered the necessity of distinguishing them from the causative agent of an erysipeletous infection, E. rhusiopathiae, to which they are very similar. This proved to be particularly important in connection with the fact that we very frequently isolated rhusiopathiae cultures from the wild rodents and insectivora. We found that it is possible to successfully differentiate between these two causative agents by a series of criteria which are presented in table 3. Our system is less detailed than the system proposed for the same purpose by Svintsov (1948). We do not include in the system a verification of the motility, upon which some authors insist. In the investigation of the strains we were able to convince ourselves that listeria has a weak independent motility, usually expressed only in young cultures. Erysipelothrix have no active motility, but due to their small size they are subjected to a rather intende Brownian movement in a suspended droplet. The differences between the active motility of listeria and the passive motility of erysipelothrix are not demonstrative and cannot be considered reliable. Orlova (1948) relates that on farms affected by swine erysipelas she isolated strains from sick and dead gilts that were completely identical by biochemical and cultural properties to the swine erysipelas pathogen, but which had a clearly expressed motility in the cultures.

(Table 3)

Conclusions

1. Listerellosis was discovered in a natural state in field mice (Microtus arvalis Pall.) - 8 finds, in a water rat (Arvicola terrestris L.) - one find, in a water shrew (Neomys fodiens Schrb.) - one find, and in a common shrew (Sorex araneus L.) - one find. One strain of L monocytogenes was isolated from pubescent ticks, Dermacentor pictus Herm., taken from healthy cows.

2. All of the isolated strains, by morphological, cultural, tinctorial, biochemical and antigenic properties, were completely similar to each other and fully corresponded to Listeria monocytogenes (Mur., Wb et Sw.).

3. The strains possessed an express pathogenicity for field mice, white mice and rabbits. For the first two types of animals the minimum lethal dose in a subcutaneous infection amounted to 10-100 thousand microbic cell.

4. The established phenomenon of natural incidence of listerellosis in rodents and insectivora, which are widely spread in nature, and also in ticks, increases the knowledge concerning the epizootology and epidemiology of this little investigated disease.

Footnotes

1. The field portion of the investigations was fulfilled in a joint expedition of the Institute of Epidemiology and Microbiology, AMN, USSR - the Moscow Antiplague Observation Station - the Moscow Institute of Epidemiology, Microbiology and Infectious Diseases imeni Mechnikov - and the Central Disinfection Institute at the Mikhnovskii Tularemia Station (the expedition's supervisor was Prof. N. G. Olsuf'ev).

2. In some cases by the 27th day, traces of acid appeared in even these two alcohols, but it is uncertain that the changes which occurred could be acknowledged as a true fermentation.

Table 1. The different dilutions of antiserum which agglutinated, in the cross-agglutination test, strains of Listeria monocytogenes of different origin.

No. of Strain	No. of Antiserum 139	81	243	449	1321	2026	2171	2753
139	1600	400	800	800	400	800	800	400
249	400	800	800	800	200	800	1600	200
81	800	1600	800	800	100	800	800	200
153	400	1600	800	800	100	800	800	800
243	800	1600	1600	800	200	1600	1600	400
285	400	1600	800	800	400	1600	800	400
449	400	1600	400	1600	800	1600	1600	400
1321	200	800	200	400	50	800	800	200
2026	800	800	1600	800	400	1600	800	400
2171	400	800	800	800	400	800	1600	200
2753	400	800	400	800	800	1600	3200	3200
3503	1600	1600	1600	1600	1600	1600	1600	800

Note: Agglutination was only taken into account for four and three crosses.

Table 2. Sensitivity of field mice and white mice to listerellosis with a subcutaneous infection.

Strain No.	Type of animal	No. of animals tested	Dose of microbic cells. 1,000	10,000	100,000	1 mln	10 mln	100 mln
285	field mouse		-p	-p	- 4	4 4	4 4	4 4
		12	23	19	5	4 4	4 3	4 3
81	" "	8			p 4	- -	4 4	4 4
					22 4		3 4	3 2
81	white mouse	10		- 4	4	p 4	- 4	4 4
				8	- 10	15 7	5	4 4
153	field mouse	9		- 4	4 4	4 4	- 4	4
				5	4 3	5 4	4	3
153	white mouse	10		- -	4	4 4	4	4 p 4
					- 6	13 5	4	3 2 3

Conventional Symbols:

4 animal died from listerellosis
p animal died from an outside cause
- animal survived
numbers indicate the times of death in 24-hour periods.

Table 3. Basic differences between Listeria monocytogenes and Erysipelothrix rhusiopathiae.

Criteria	Listeria monocytogenes	Erysipelothrix rhusiopathiae
Morphology from an agar culture	Straight, short ovoid rods of a 0.3-0.5 micron thickness.	Often the more or less curved rods are of a 0.1-0.3 micron thickness (approx. two times narrower than listeria.
A 24-hour growth on a slant, weakly alkaline meat-peptone agar.	The growth along the streak and the separate colonies are easily visible with the naked eye.	The growth along the streak and the separate colonies are hardly visible with the naked eye (the colonies are easily seen in a magnifying glass with an enlargement of × 10 and more).
Growth on a meat-peptone gelatin culture with a puncture.	Small knots - colonies along the puncture; in old cultures sometimes in the upper portion of the puncture are small fluffy outgrowths.	Fluffy offshoots running perpendicularly to the puncture along its entire length (lamp brush).
Fermentation of carbohydrates.	Within 24 hours it breaks down to acids glucose and maltose, later, lactose, sucrose and glycerol.	Within 24-48 hours it breaks lactose and glucose to an acid. Some strains break down sucrose (variant muriseptica). It does not ferment maltose and glycerol.
Reaction of methyl red on a Clark medium after a 96-hour growth at 37°.	Positive (reddening of the culture medium).	Negative (yellowing of the culture medium).
Antigenic properties.	Agglutinates only with homologous antiserums.	Agglutinates with homologous antiserums and some strains agglutinate with antilisteria serums in small dilutions.